IDENTIFICATION AND CHARACTERIZATION OF POLYTENE CHROMOSOMES OF *Bactrocera papayae* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The present study constitutes a first attempt to identify the larval salivary gland polytene chromosome arms of *Bactrocera papayae* Drew & Hancock. This species belongs to the family Tephritidae and is commonly known as the Asian papaya fruit fly. A photographic representation of the polytene chromosomes of this species is provided and the identifying tips as well as most prominent landmarks of each polytene chromosome are presented and discussed. Each polytene nucleus consists of five long chromosomes. According to the position of centromere, the longer arm was designated as left (L) and the shorter arm as right (R). No polytenized sex chromosomes are seen, indicating that five polytene chromosomes correspond to the five autosomes of mitotic chromosomes.

Key words: fruit fly, *Bactrocera papayae*, polytene chromosomes, salivary gland, polyphagous pest

INTRODUCTION

Fruit flies (Tephritidae) are a serious economic pest affecting horticultural production world-wide. There are over 4,000 species in this family and more than 800 species in the sub-family Dacinae, which are the main species that infest soft fruits in tropical and sub-tropical areas (Bellas 1996). The *Bactrocera dorsalis* complex contains 75 described species, largely endemic to Southeast Asia. Within this complex a small number of polyphagous pests have international significance. This includes the Asian papaya fruit fly, *Bactrocera papayae* Drew & Hancock (Drew & Hancock 1994). In Malaysia, there are possibly at least a hundred *Bactrocera* species, of which only about half have been recorded (Chua 1998). Of these, the melon fly, *B. cucurbitae*, the papaya fruit fly, *B. papayae* Drew & Hancock, the carambola fruit fly, *B. carambolae* Drew & Hancock, the nangka fruit fly, *B. umbrosa* Fabricius, and the Malaysian fruit fly, *B. latifrons* Hendel, are major agricultural pests (Chua *et al*., 2010). Fruit fly pests feed and breed around their host plants and lay eggs in the ripening fruit (Drew & Romig 1997). When the larvae emerge, they feed off the ripening fruit. This can cause fruit to drop prior to harvest, or if harvested, the fruit cannot be sold. In some fruits, losses can be very high. Tobin (1990) reported that losses close to 100% in carambola and guava plantings in Malaysia due to fruit fly pests without specifying the species. Because of the potential losses, fruit fly control is typically carried out in commercial plantings. It involves application of chemical insecticides. The intensive use of sprays in fruit crops can raise growers' risk of exposure and the potential for long term health problems (Ferrar 1988). However, there is considerable efforts in genetic control techniques which are environment-friendly and species-specific, such as the sterile insect technique (SIT), sterile male technique etc. (Zhao *et al.*, 1998). An understanding of the genetics of this species is essential for the development of novel control methods (Mavragani-Tsipidou 2002).

In many Diptera, polytene chromosomes have proved to be very useful for cytogenetic and genetic studies (Sorsa 1988). The information is needed for monitoring chromosome rearrangements as markers

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of the population composition of the target species (Mavragani-Tsipidou 1992). To conduct this work, good cytology is an essential aid for examining chromosome structure, behavior and function (Bedo 1986). Workable polytene chromosomes are especially favorable for autosomal studies allowing accurate analysis of different chromosome regions (Augustinos *et al.* 2011). At present, polytene chromosomes of more than 270 drosophilid species and about 250 other dipteran species are studied worldwide (Drosopoulou *et al.* 2012). *Bactrocera papayae* belongs to a large group known as *Bactrocera dorsalis* complex. Until now, the polytene chromosomes of *B. dorsalis* (Hendel) of this complex have been studied by Zacharopoulou *et al.* (2011b).

In this paper, the polytene chromosomes of the larval salivary glands of *B. papayae* are described for the first time. The purpose is to present basic genetic information of this pest that may be helpful for species discrimination and lead to the development of an environmentally friendly control policy.

MATERIALS AND METHODS

Fly stock

Initial cultures of *Bactrocera papayae* was collected from the Malaysian Agricultural Research and Development Institute (MARDI), Serdang in July 2010. From this initial culture, the laboratory populations are established and maintained in the UKM laboratory, Bangi. Currently the lab cultures are up to 14th generation. Cultures are maintained at 25±1ºC with 70±5% relative humidity and a 14 h light- 10 h dark cycle. Adults are fed on a mixture of sugar and yeast (3:1). Larvae are grown on sweet gourd medium.

Polytene chromosome preparations

Third-instar larvae (6-7 days old) were used for the salivary gland polytene chromosome preparations following the method described by Shahjahan & Yesmin (2002) with slight modifications. At least one thousand larvae were studied. Larval salivary glands were dissected in 45% acetic acid and then fixed in 3N HCL for 1-2 min. The material was transferred to a drop of 3:2:1 solution (acetic acid: distilled water: lactic acid) for 5 min and stained in lactoacetic orcein for about 15-20 min. Then the tissues were squashed in the 3:2:1 solution mentioned above. The slides were examined using an Olympus BX41 microscope and the clear and well-distributed polytene chromosome nuclei were photographed with Soft Imaging System GmbH 5.0.1054. Well-spread chromosomes with clear banding patterns were photographed and described.

The chromosomes were identified, numbered and analyzed as it is commonly done for the polytene chromosomes of fruit fly species (Zacharopoulou 1987; Shahjahan *et al.*, 2000; Shahjahan & Yesmin 2002; Zacharopoulou *et al.*, 2011a). The identifying tips of each chromosome arm are detected. Efforts are also carried out to locate the characteristic features and landmarks of different chromosome arms. According to the position of the centromere, the two arms in each chromosome are of unequal length. The longer arm was designated as left (L) and the shorter arm as right (R). Two criteria were used for the location of the centromere in each arm: (i) in unbroken chromosome, the centromere presented a point of discontinuity, and (ii) in case of broken chromosomes, the centromere was the frequent point of fragmentation (Zacharopoulou 1987; Shahjahan & Yesmin 2002).

RESULTS AND DISCUSSION

The analysis of the salivary gland polytene chromosomes showed that the *B. papayae* polytene complement consists of a total of five long chromosomes (10 polytene arms). The photographic chromosomes are given in Figure 1-5. A brief account of the characteristics and prominent landmarks of each polytene chromosome is given below.

Chromosome 2: The left and right arms of chromosome 2 are usually connected to the heterochromatic mass in their centromeric region (Figure 1a,b). There are several weak points (Fig. 1b, indicated as W) in 2L arm which is a common characteristic of the left arm of chromosome 2 of other fruit fly spp. In addition, extensive ectopic pairing sometimes creates folding or twisting appearance of this arm (Fig. 1a, indicated by close arrow), making it difficult to analyze the banding pattern along the entire arm's length. The distal region 2LA is the easily analyzed region of the left arm (Fig. 1b). The right arm has a comparatively good banding pattern than left arm. It can be easily recognized by its triangular tip (Fig. 1a-c) with a dark band (2RA) and the presence of a constriction in 2RB. Other identifying landmarks are 2RC region with a group of dark bands (Fig. 1a-d) and two unique shape prominent puffs in 2RD region (Fig. 1d).

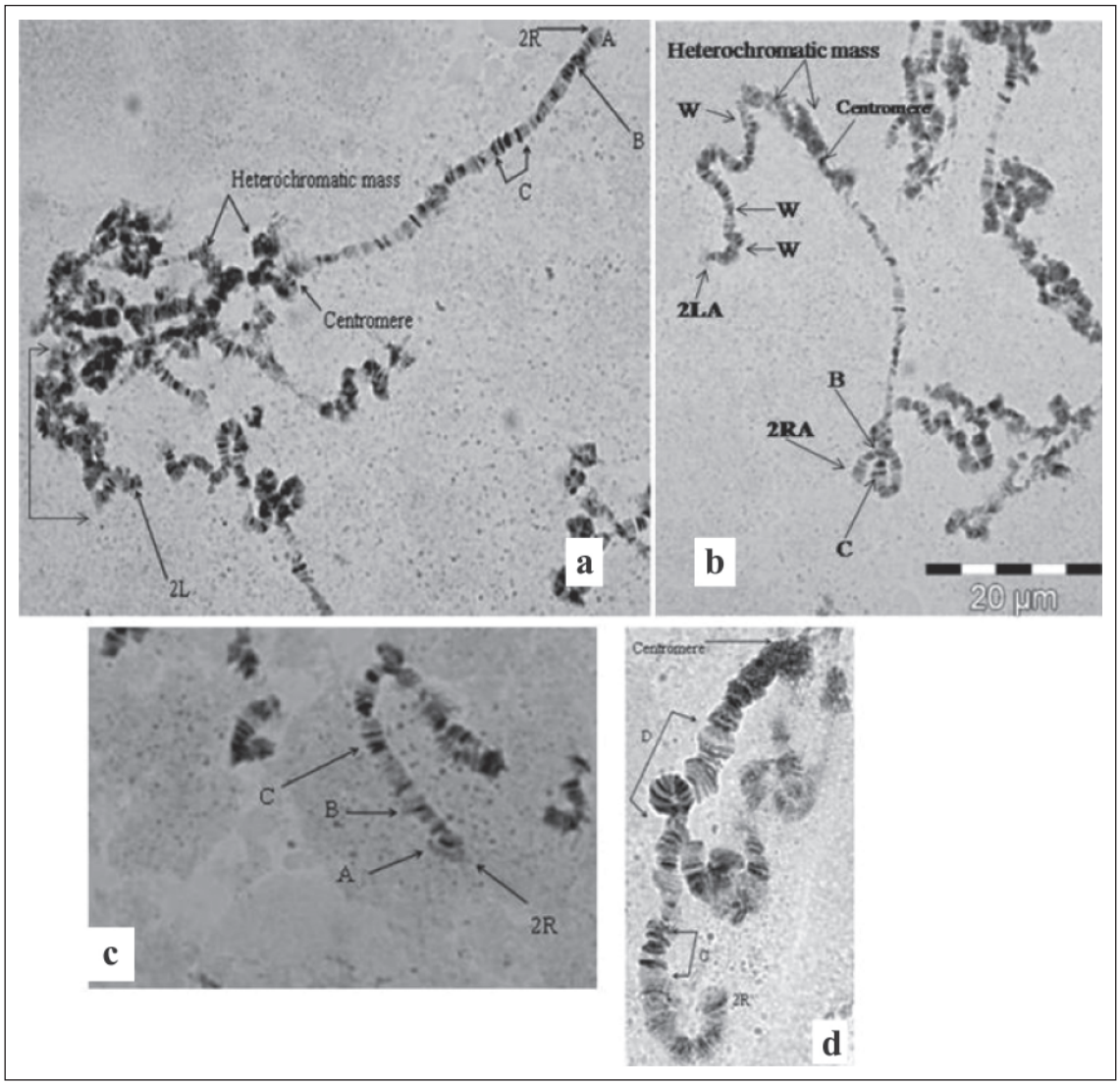

Fig. 1. Polytene chromosome 2 (a-d). L= left arm, R= right arm, W= weak points to breakage during slide preparation. Arrows indicate the identifying characteristics of chromosome arms.

Chromosome 3: The 3L tip is readily recognized by the presence of dotted dark band followed by an expanded region, 3LA (Fig. 2a-d). Another important landmark is a unique puff in 3LB (Fig 2b,c). The right arm was difficult to identify. Very few nuclei were found with this arm and could not be separated well (Fig. 2a,d).

Chromosome 4: This chromosome is relatively easy to identify by a number of distinctive appearance. The 4L tip is characterized by square shape with two close dark bands (Fig. 3). The 4R tip is also square shaped and consists of a number of thin bands. Regions A, C, D are important landmarks of the right arm. The presence of a constriction at the right arm, 4RB serves as an identifying tip of this chromosome (Fig. 3).

Chromosome 5: This chromosome is easily found in every polytene nucleus. Two tips, 5L and 5R are usually seen as free ends, especially the right tip (Fig. 4). 5L is much longer than 5R. The tip of left arm is swollen with dark bands and presence of puffs in 5LA region are important features of this arm (Fig. 4a,c). The right arm has a very clear banding pattern. In fact, whole 5R arm (Fig.4, indicated by close arrows) serves as diagnostic features in the complement with a characteristic region in 5RB (Fig. 4a-e). The complete right arm of chromosome 5 with centromere is shown in Fig. 4b,d.

Chromosome 6: The left arm of this chromosome is longer than the right arm, a similar state to chromosome 5. Both end of this chromosome are also free and the left tip is composed of thin bands (6LA). The presence of two puffs in left arm (6LB) and a prominent puff (6RC) in right telomeric region are the identifying landmarks of this arm.

Bactrocera papayae is a sibling species of *Bactrocera dorsalis* complex (Clarke *et al.*, 2005).

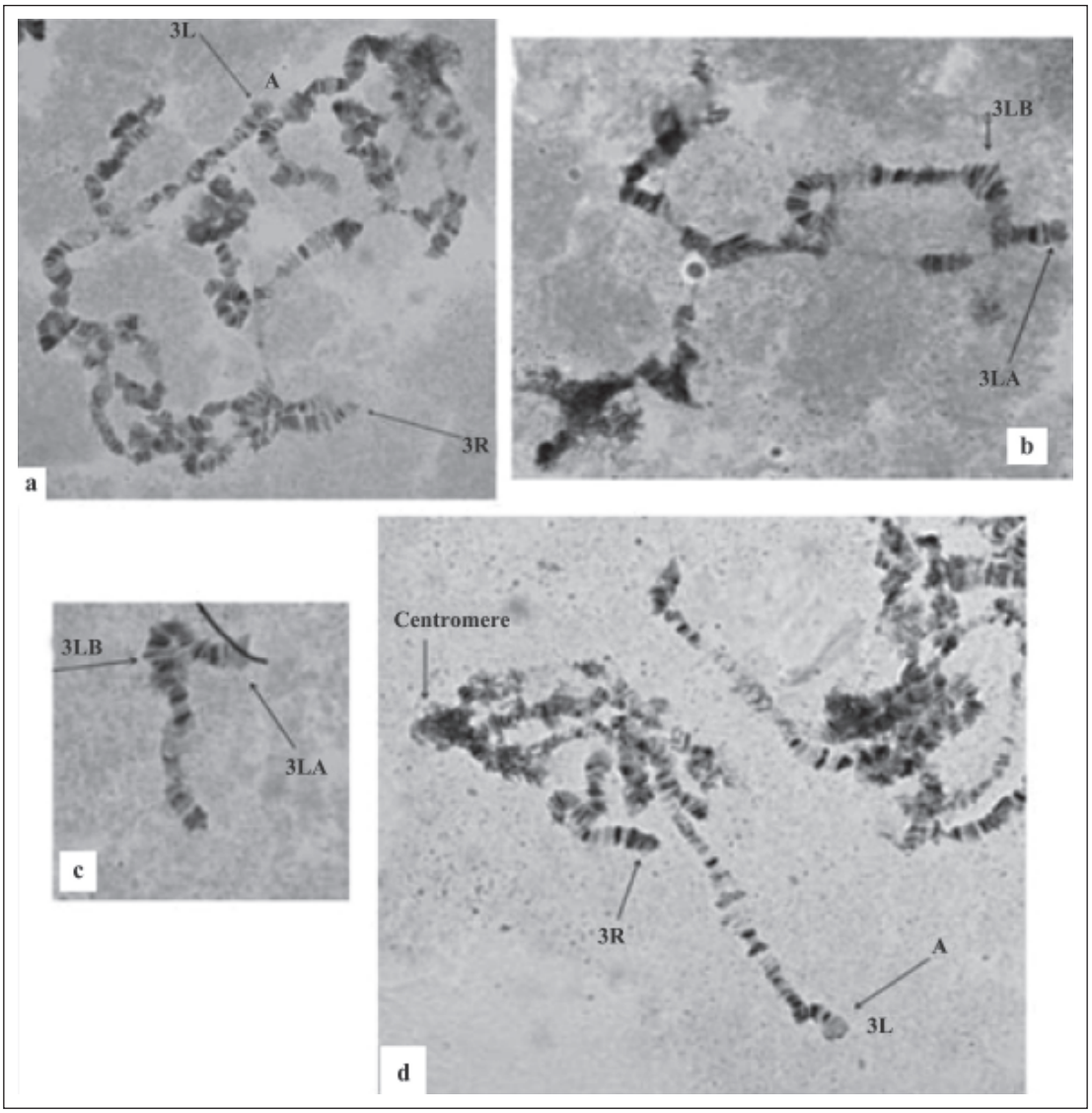

Fig. 2. Polytene chromosome 3 (a-d). L= left arm, R= right arm. Arrows indicate the identifying characteristics of chromosome arms.

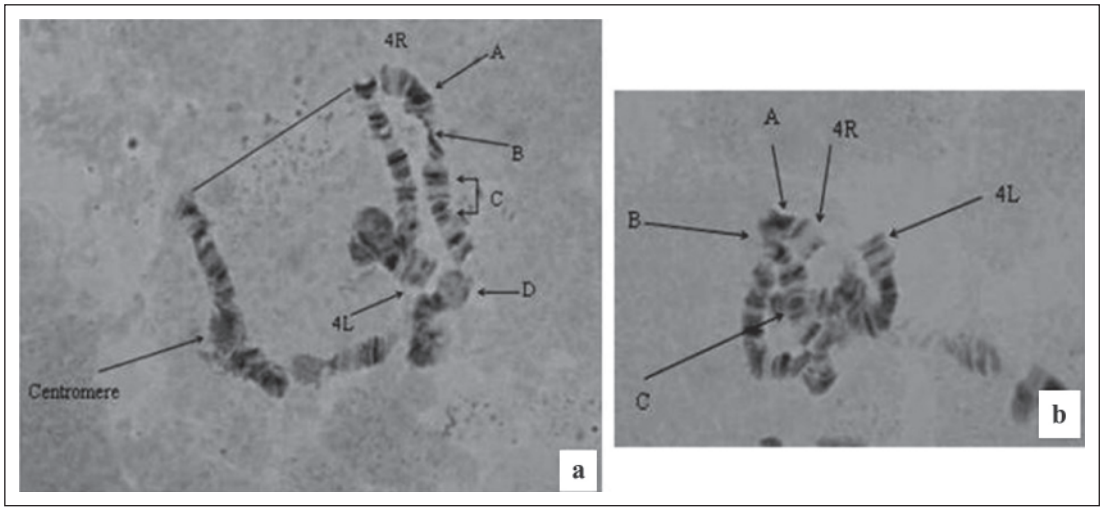

Fig. 3. Polytene chromosome 4 (a, b). L= left arm, R= right arm. Arrows indicate the identifying characteristics of chromosome arms.

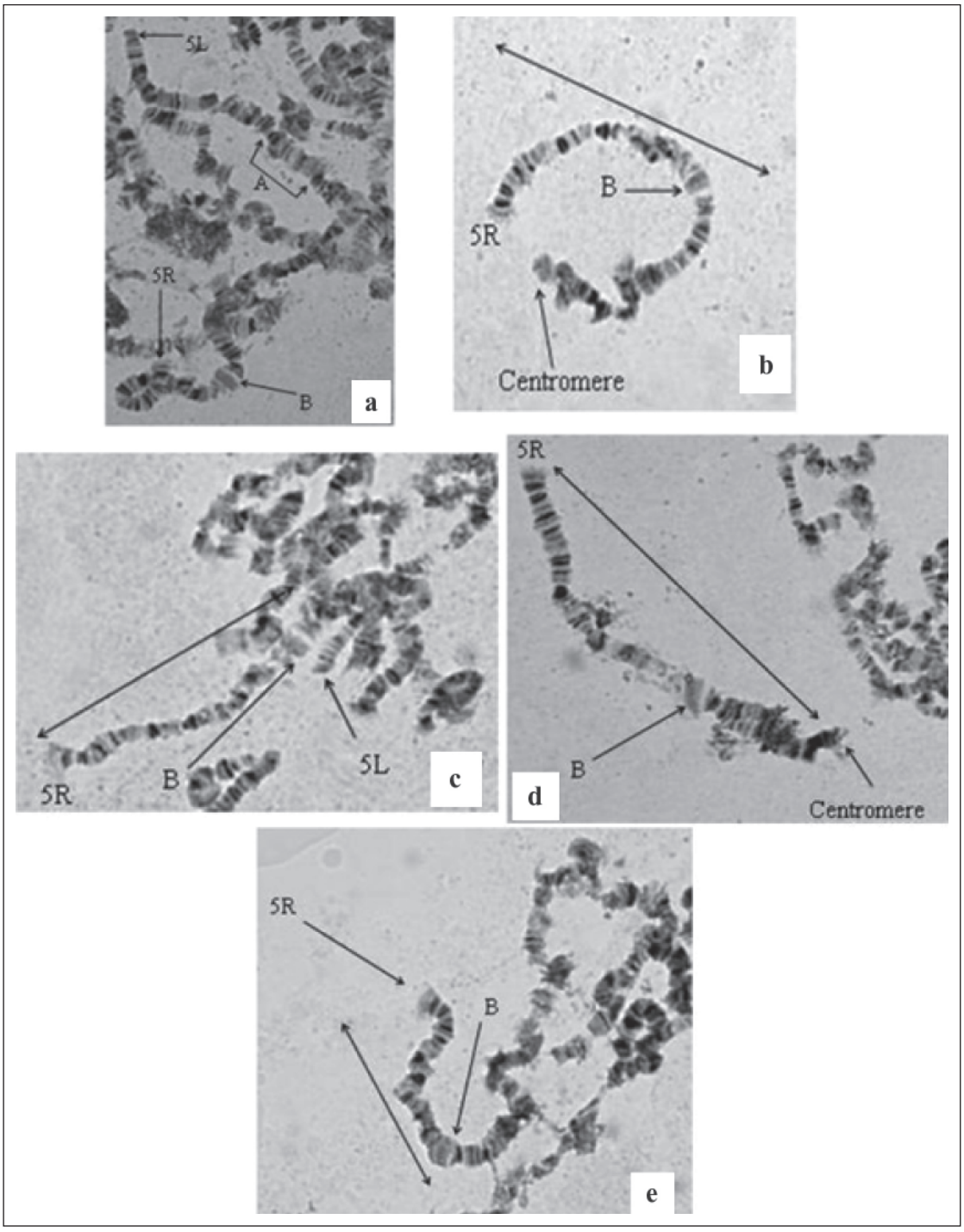

Fig. 4. Polytene chromosome 5 (a-e). L= left arm, R= right arm. Arrows indicate the identifying characteristics of chromosome arms.

At present, polytene chromosomes of *B. dorsalis* (Hendel) have been reported within this group (Zacharopoulou *et al.*, 2011b). They reported five banded polytene chromosomes in the salivary gland nuclei. Our findings are consistent with this report and also with other *Bactrocera* species, melon fly, *Bactrocera cucurbitae* (Shahjahan *et al.*, 2000; Shahjahan & Yesmin 2002; Zacharopoulou *et al.*, 2011a), the Queensland fruit fly, *Bactrocera tryoni* (Zhao *et al.*, 1998) and olive fruit fly, *Bactrocera oleae* (Zambetaki *et al.*, 1995). In the polytene chromosome preparations, we did not observe any difference between males and females. This indicates the absence of polytenization of both X and Y chromosomes. So the five polytene chromosomes correspond to the five autosomes of the mitotic nuclei. This species has six pairs of mitotic chromosomes, including one pair of sex

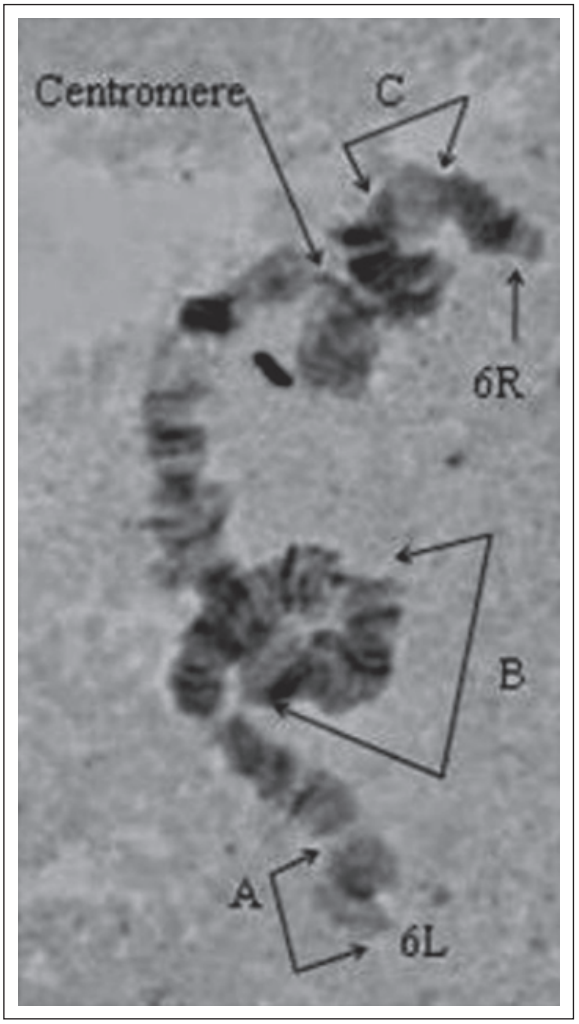

Fig. 5. Polytene chromosome 6. L= left arm, R= right arm. Arrows indicate the identifying characteristics of chromosome arms.

chromosomes (Chromosome 1), with the male being the heterogametic sex, XY (Fig. 6). This is in full agreement with previously reported Tephritid species where both sex chromosomes do not form polytene elements (Bedo 1986; Zacharopoulou 1990, 2011b; Mavragani-Tsipidou *et al.*, 1992; Zhao *et al.*, 1998; Kounatidis *et al.*, 2008; Garcia-Martinez *et al.*, 2009; Drosopoulou *et al.*, 2010, 2012). Also it is known that the heterochromatic chromosomes are not polytenized or under-replicated in polytene nuclei (Rudkin 1972).

Cytogenetic analysis of tephritid pest species has been greatly facilitated by the presence of polytene chromosomes, which have proven to be excellent experimental material for studying chromosome structure and function together with mitotic chromosomes. The identification and characterization of the salivary gland polytene chromosomes of this important pest species needs to be completed by a detailed chromosome mapping and by a correlation with the mitotic chromosomes. Such a work is currently in progress under the chromosomal study of fruit fly project.

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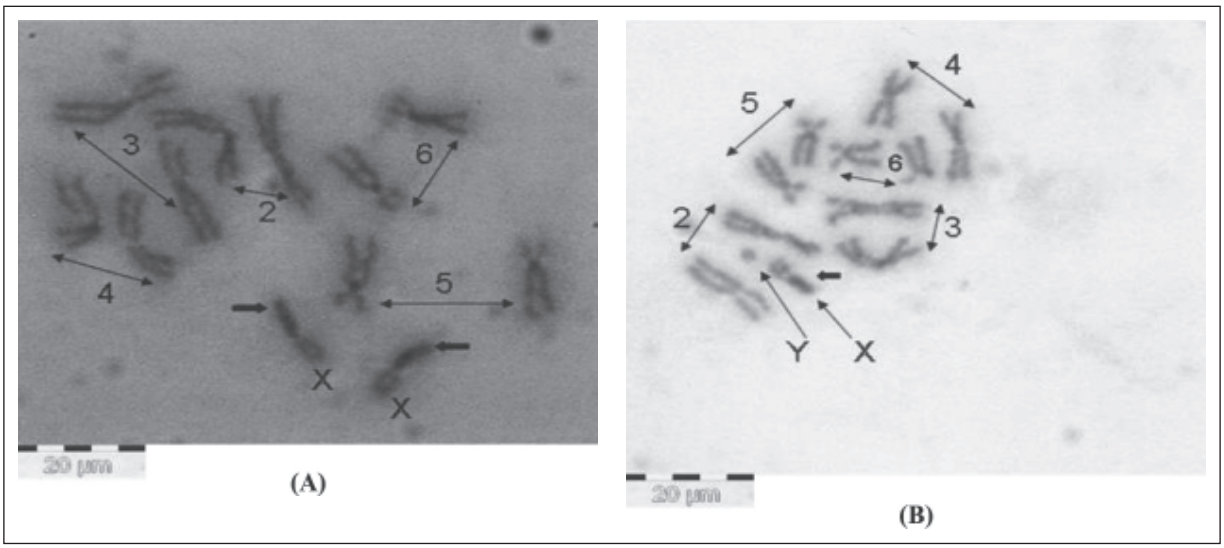

Fig. 6. Mitotic metaphase chromosomes from brain ganglia of third-instar larvae of *Bactrocera papayae*. Homologous chromosomes show somatic pairing (indicated by arrow lines at both ends). Block arrows show the heterochromatic arm of X chromosome. (A) Female karyotype. (B) Male karyotype. (Source: Yesmin & Clyde 2012)

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